Table 1. Sequence of LoxP Sites

5'-ATAACTTCGTATAATGTATGCTATACGAAGTTAT-3' LoxP WT [SEQ ID NO: 1] **LoxP511** 5'-ATAACTTCGTATAGTATACATTATACGAAGTTAT-3' [SEQ ID NO: 2] LoxC2 5'-ACAACTTCGTATAATGTATGCTATACGAAGTTAT-3' [SEQ ID NO: 3] 5'-ATAACTTCGTATAATATATGCTATACGAAGTTAT-3' [SEQ ID NO: 4] 5'-ATAACTTCGTATAGCATACATTATACGAAGTTAT-3' [SEQ ID NO: 5] 5'-ATAACTTCGTATAATGTATACTATACGAAGTTAT-3' LoxP3 [SEQ ID NO: 6] LoxP4 5'-ATAACTTCGTATAATATAAACTATACGAAGTTAT-3' [SEQ ID NO: 7] LoxP5 5'-ATAACTTCGTATAATCTAACCTATACGAAGTTAT-3' [SEQ ID NO: 8] LoxP6 5'-ATAACTTCGTATAACATAGCCTATACGAAGTTAT-3' [SEQ ID NO: 9] LoxP7 5'-ATAACTTCGTATAACATACCCTATACGAAGTTAT-3' [SEQ ID NO: 10] LoxP8 5'-ATTACCTCGTATAGCATACATTATACGAAGTTAT-3' [SEQ ID NO: 11] LoxP9 5'-ATAACTTCGTATAGCATACATTATATGAAGTTAT-3' [SEQ ID NO: 12] **LoxP10** 5'-ATTACCTCGTATAGCATACATTATATGAAGTTAT-3' [SEQ ID NO: 13]

(130)

Table 2. Sequence of PCR primers for amplifying heavyand light-chain genes of human antibody.

(B= C/G/T; D= A/G/T; K= G/T; M= A/C; R= A/G; S= C/G; W= A/T; and Y= C/T)

a) Heavy-chain VH

5'-primers (back primers):

VH1b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT TAC CCA TAC GAT GTT CCA GAT TAC CAG GTG CAG CTG CAG GAG TCS G-3' [SEQ ID NO: 14]

VH2b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT TAC CCA TAC GAT GTT CCA GAT TAC CAG GTA CAG CTG CAG CAG TCA-3' [SEQ ID NO: 15]

VH3b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT TAC CCA TAC GAT GTT CCA GAT TAC CAG GTG CAG CTA CAG CAG TGG G-3' [SEQ ID NO: 16]

VH4b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT TAC CCA TAC GAT GTT CCA GAT TAC GAG GTG CAG CTG KTG GAG WCY-3' [SEQ ID NO: 17]

VH5b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT TAC CCA TAC GAT GTT CCA GAT TAC CAG GTC CAG CTK GTR CAG TCT GG-3' [SEQ ID NO: 18]

VH6b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT TAC CCA TAC GAT GTT CCA GAT TAC CAG RTC ACC TTG AAG GAG TCT G-3' [SEQ ID NO: 19]

VH7b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT TAC CCA TAC GAT GTT CCA GAT TAC CAG GTG CAG CTG GTG SAR TCT GG-3' [SEQ ID NO: 20]

3'-primers (forward primers):

VH1f: 5'-ACT GCC TCC ACC ACC GCT GCC ACC TCC GCC AGA TCC TCC GCC GCC TGA TCC ACC ACC GCC TGA GAC RGT GAC CAG GGT G-3' [SEQ ID NO: 21]

VH2f: 5'-ACT GCC TCC ACC ACC GCT GCC ACC TCC GCC AGA TCC
TCC GCC GCC TGA TCC ACC ACC GCC TGA GAC GGT GAC CAG
GGT T-3' [SEQ ID NO: 22]

VH3f: 5'-ACT GCC TCC ACC ACC GCT GCC ACC TCC GCC AGA TCC TCC GCC GCC TGA TCC ACC ACC GCC TGA AGA GAC GGT GAC CAT TGT-3' [SEQ ID NO: 23]

VH4f: 5'-ACT GCC TCC ACC ACC GCT GCC ACC TCC GCC AGA TCC
TCC GCC GCC TGA TCC ACC ACC GCC TGA GAC GGT GAC CGT
GGT CC-3' [SEQ ID NO: 24]

VH5f: 5'-ACT GCC TCC ACC ACC GCT GCC ACC TCC GCC AGA TCC TCC GCC GCC TGA TCC ACC ACC GCC GGT TGG GGC GGA TGC ACT CC-3' [SEQ ID NO: 25]

VH6f: 5'-ACT GCC TCC ACC ACC GCT GCC ACC TCC GCC AGA TCC TCC GCC GCC TGA TCC ACC ACC GCC SGA TGG GCC CTT GGT GGA RGC-3' [SEQ ID NO: 26]

b) Light-chain $V\lambda$

5'-primers (back primers):

Vλ1b: 5'-GGC GGT GGT GGA TCA GGC GGA GGA TCT GGC GGA
GGT GGC AGC GGT GGA GGC AGT CAG TCT GTS BTG ACG CAG
CCG CC-3' [SEQ ID NO: 27]

Vλ2b: 5'-GGC GGT GGT GGA TCA GGC GGC GGA GGA TCT GGC GGA GGT GGC AGC GGT GGA GGC AGT TCC TAT GWG CTG ACW CAG CCA C-3' [SEQ ID NO: 28]

Vλ3b: 5'-GGC GGT GGT GGA TCA GGC GGA GGA TCT GGC GGA GGT GGC AGC GGT GGA GGC AGT TCC TAT GAG CTG AYR CAG CYA CC-3' [SEQ ID NO: 29]

Vλ4b: 5'-GGC GGT GGT GGA TCA GGC GGC GGA GGA TCT GGC GGA GGT GGC AGC GGT GGA GGC AGT CAG CCT GTG CTG ACT CAR YC-3' [SEQ ID NO: 30]



Vλ6b: 5'-GGC GGT GGT GGA TCA GGC GGC GGA GGA TCT GGC GGA GGT GGC AGC GGC AGC CCW GKG CTG ACT CAG CCM CC-3' [SEQ ID NO: 32]

Vλ7b: 5'-GGC GGT GGT GGA TCA GGC GGC GGA GGA TCT GGC GGA GGT GGC GGC GGC AGC GGC AGC GGC AGC CTG AST CAG GAS CC-3' [SEQ ID NO: 33]

3'-primers (forward primers):

Vλ1f: 5'-GAG ATG GTG CAC GAT GCA CAG TTG AAG TGA ACT TGC GGG GTT TTT CAG TAT CTA CGA TTC TAG GAC GGT SAS CTT GGT CC-3' [SEQ ID NO: 36]

Vλ2f: 5'-GAG ATG GTG CAC GAT GCA CAG TTG AAG TGA ACT TGC GGG GTT TTT CAG TAT CTA CGA TTC GAG GAC GGT CAG CTG GGT GC-3' [SEQ ID NO: 37]

c) Light-chain VK

5'-primers (back primers):

VK4b: 5'-GGC GGT GGT GGA TCA GGC GGC GGA GGA TCT GGC GGA GGT GGC AGC AGC ACC ACC CAG TCT C-3' [SEQ ID NO: 41]

3'-primers (forward primers):

Vk1f: 5'-GAG ATG GTG CAC GAT GCA CAG TTG AAG TGA ACT TGC GGG GTT TTT CAG TAT CTA CGA TTC TTT GAT TTC CAC CTT GGT CC-3' [SEQ ID NO: 42]

Vk2f: 5'-GAG ATG GTG CAC GAT GCA CAG TTG AAG TGA ACT TGC GGG GTT TTT CAG TAT CTA CGA TTC TTT GAT CTC CAS CTT GGT CC-3' [SEQ ID NO: 43]

Vk3f: 5'-GAG ATG GTG CAC GAT GCA CAG TTG AAG TGA ACT TGC GGG GTT TTT CAG TAT CTA CGA TTC TTT GAT ATC CAC TTT GGT CC-3' [SEQ ID NO: 44]

VK4f: 5'-GAG ATG GTG CAC GAT GCA CAG TTG AAG TGA ACT TGC GGG GTT TTT CAG TAT CTA CGA TTC TTT AAT CTC CAG TCG TGT CC-3' [SEQ ID NO: 45]



Table 3. Sequence of oligonucleotides for modifying the cloning vector pACT2.

a) The sense strand

5'-TCGA GGC GGT GGT GGA TCA GGC GGC GGA GGA TCT GGC GGA GGT GGC AGC GGT GGT GGA GGC AGT GCG CGC TTA ATT AA-3' [SEQ ID NO: 46]

b) The antisense strand

5'-TCG ATT AAT TAA GCG CGC ACT GCC TCC ACC ACC GCT GCC ACC TCC GCC AGA TCC TCC GCC GCC TGA TCC ACC ACC GCC-3' [SEQ ID NO: 47]

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Table 4. Sequence of PCR primers that include loxP sites for amplifying heavy- and light-chain genes of human antibody.

(B= C/G/T; D= A/G/T; K= G/T; M= A/C; R= A/G; S= C/G; W= A/T; and Y= C/T)

a) Heavy-chain VH

5'-primers (back primers):

VH1b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT TAC CCA TAC GAT GTT CCA GAT TAC CAG GTG CAG CTG CAG GAG TCS G-3' [SEQ ID NO: 14]

VH2b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT TAC CCA TAC GAT GTT CCA GAT TAC CAG GTA CAG CTG CAG CAG TCA-3' [SEQ ID NO: 15]

VH3b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT TAC CCA TAC GAT GTT CCA GAT TAC CAG GTG CAG CTA CAG CAG TGG G-3' [SEQ ID NO: 16]

VH4b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT TAC CCA TAC GAT GTT CCA GAT TAC GAG GTG CAG CTG KTG GAG WCY-3' [SEQ ID NO: 17]

VH5b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT TAC CCA TAC GAT GTT CCA GAT TAC CAG GTC CAG CTK GTR CAG TCT GG-3' [SEQ ID NO: 18]

VH6b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT TAC CCA TAC GAT GTT CCA GAT TAC CAG RTC ACC TTG AAG GAG TCT G-3' [SEQ ID NO: 19]

VH7b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT TAC CCA TAC GAT GTT CCA GAT TAC CAG GTG CAG CTG GTG SAR TCT GG-3' [SEQ ID NO: 20]

3'-primers (forward primers):

VH1'f: 5'-ACT GCC TCC ACC TGA TAA CTT CGT ATA GCA TAT ATT ATA CGA AGT TAT TGA TCC ACC ACC GCC TGA GGA GAC RGT GAC CAG GGT G-3' [SEQ ID NO: 48]

- VH2'f: 5'-ACT GCC TCC ACC TGA TAA CTT CGT ATA GCA TAT ATT ATA CGA AGT TAT TGA TCC ACC ACC GCC TGA GGA GAC GGT GAC CAG GGT T-3' [SEQ ID NO: 49]
- VH3'f: 5'-ACT GCC TCC ACC TGA TAA CTT CGT ATA GCA TAT ATT ATA CGA AGT TAT TGA TCC ACC ACC GCC TGA AGA GAC GGT GAC CAT TGT-3' [SEQ ID NO: 50]
- VH4'f: 5'-ACT GCC TCC ACC TGA TAA CTT CGT ATA GCA TAT ATT ATA CGA AGT TAT TGA TCC ACC ACC GCC TGA GGA GAC GGT GAC CGT GGT CC-3' [SEQ ID NO: 51]
- VH5'f: 5'-ACT GCC TCC ACC TGA TAA CTT CGT ATA GCA TAT ATT ATA CGA AGT TAT TGA TCC ACC ACC GCC GGT TGG GGC GGA TGC ACT CC-3' [SEQ ID NO: 52]
- VH6'f: 5'-ACT GCC TCC ACC TGA TAA CTT CGT ATA GCA TAT ATT ATA CGA AGT TAT TGA TCC ACC ACC GCC SGA TGG GCC CTT GGT GGA RGC-3' [SEQ ID NO: 53]

b) Light-chain $V\lambda$

5'-primers (back primers):

Vλ1'b: 5'-GGC GGT GGT GGA TCA ATA ACT TCG TAT AAT ATA TGC TAT ACG AAG TTA TCA GGT GGA GGC AGT CAG TCT GTS BTG ACG CAG CCG CC-3' [SEQ ID NO: 54]

V λ 2'b: 5'-GGC GGT GGT GGA TCA ATA ACT TCG TAT AAT ATA TGC TAT ACG AAG TTA TCA GGT GGA GGC AGT TCC TAT GWG CTG ACW CAG CCA C-3' [SEQ ID NO: 55]

Vλ3'b: 5'-GGC GGT GGT GGA TCA ATA ACT TCG TAT AAT ATA TGC

TAT ACG AAG TTA TCA GGT GGA GGC AGT TCC TAT GAG CTG AYR

CAG CYA CC-3' [SEQ ID NO: 56]

Vλ4'b: 5'-GGC GGT GGT GGA TCA ATA ACT TCG TAT AAT ATA TGC TAT ACG AAG TTA TCA GGT GGA GGC AGT CAG CCT GTG CTG ACT CAR YC-3' [SEQ ID NO: 57]

Vλ5'b: 5'-GGC GGT GGT GGA TCA ATA ACT TCG TAT AAT ATA TGC TAT ACG AAG TTA TCA GGT GGA GGC AGT CAG DCT GTG GTG ACY CAG GAG CC-3' [SEQ ID NO: 58]

Vλ6'b: 5'-GGC GGT GGT GGA TCA ATA ACT TCG TAT AAT ATA TGC

TAT ACG AAG TTA TCA GGT GGA GGC AGT CAG CCW GKG CTG ACT

CAG CCM CC-3' [SEQ ID NO: 59]

Vλ7'b: 5'-GGC GGT GGT GGA TCA ATA ACT TCG TAT AAT ATA TGC TAT ACG AAG TTA TCA GGT GGA GGC AGT TCC TCT GAG CTG AST CAG GAS CC-3' [SEQ ID NO: 60]

Vλ8'b: 5'-GGC GGT GGT GGA TCA ATA ACT TCG TAT AAT ATA TGC
TAT ACG AAG TTA TCA GGT GGA GGC AGT CAG TCT GYY CTG AYT
CAG CCT-3' [SEQ ID NO: 61]

VA9'b: 5'-GGC GGT GGT GGA TCA ATA ACT TCG TAT AAT ATA TGC
TAT ACG AAG TTA TCA GGT GGA GGC AGT AAT TTT ATG CTG ACT
CAG CCC C-3' [SEQ ID NO: 62]

3'-primers (forward primers):

Vλ1'f: 5'-CTT CGT ATA ATG TAT GCT ATA CGA AGT TAT TAG GAC GGT SAS CTT GGT CC-3' [SEQ ID NO: 63]

Vλ2'f: 5'-CTT CGT ATA ATG TAT GCT ATA CGA AGT TAT GAG GAC GGT CAG CTG GGT GC-3' [SEQ ID NO: 64]

c) Light-chain VK

5'-primers (back primers):

VK1'b: 5'-GGC GGT GGT GGA TCA ATA ACT TCG TAT AAT ATA TGC
TAT ACG AAG TTA TCA GGT GGA GGC AGT GAC ATC CRG DTG ACC
CAG TCT CC-3' [SEQ ID NO: 65]

VK2'b: 5'-GGC GGT GGT GGA TCA ATA ACT TCG TAT AAT ATA TGC
TAT ACG AAG TTA TCA GGT GGA GGC AGT GAA ATT GTR WTG ACR
CAG TCT CC-3' [SEQ ID NO: 66]

VK3'b: 5'-GGC GGT GGT GGA TCA ATA ACT TCG TAT AAT ATA TGC
TAT ACG AAG TTA TCA GGT GGA GGC AGT GAT ATT GTG MTG ACB
CAG WCT CC-3' [SEQ ID NO: 67]

VK4'b: 5'-GGC GGT GGT GGA TCA ATA ACT TCG TAT AAT ATA TGC
TAT ACG AAG TTA TCA GGT GGA GGC AGT GAA ACG ACA CTC ACG
CAG TCT C-3' [SEQ ID NO: 68]

3'-primers (forward primers):

Vk1'f: 5'-CTT CGT ATA ATG TAT GCT ATA CGA AGT TAT TTT GAT TTC CAC CTT GGT CC-3' [SEQ ID NO: 69]

Vk2'f: 5'-CTT CGT ATA ATG TAT GCT ATA CGA AGT TAT TTT GAT CTC CAS CTT GGT CC-3' [SEQ ID NO: 70]

VK3'f: 5'-CTT CGT ATA ATG TAT GCT ATA CGA AGT TAT TTT GAT ATC CAC TTT GGT CC-3' [SEQ ID NO: 71]

VK4'f: 5'-CTT CGT ATA ATG TAT GCT ATA CGA AGT TAT TTT AAT CTC CAG TCG TGT CC-3' [SEQ ID NO: 72]

3'-primers (forward primers) for 2^{nd} PCR of $V\lambda$ and $V\kappa$:

Vλ/Vκf: 5'-GAG ATG GTG CAC GAT GCA CAG TTG AAG TGA ACT TGC GGG GTT TTT CAG TAT CTA CGA TAA CTT CGT ATA ATG TAT GCT-3' [SEQ ID NO: 73]

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| sgatgggccc ttggtggarg c | 81 |
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| cagtctgtsb tgacgcagcc gcc | 83 |
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| ggcggtggtg gatcaggcgg cggaggatct ggcggaggtg gcagcggtgg | tggaggcagt | 60 |
| cagdctgtgg tgacycagga gcc | | 83 |
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| <210> 35 <211> 82 <212> DNA <213> Artific | cial Seque | nce | | | | |
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| <210> 36 <211> 80 <212> DNA <213> Artific | cial Seque | nce . | | | | |
| <220> <223> Descrip | otion of A | rtificial S | Sequence: PC | CR primer | | |
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| gacatcorgd tgaccoagto too | | 83 |
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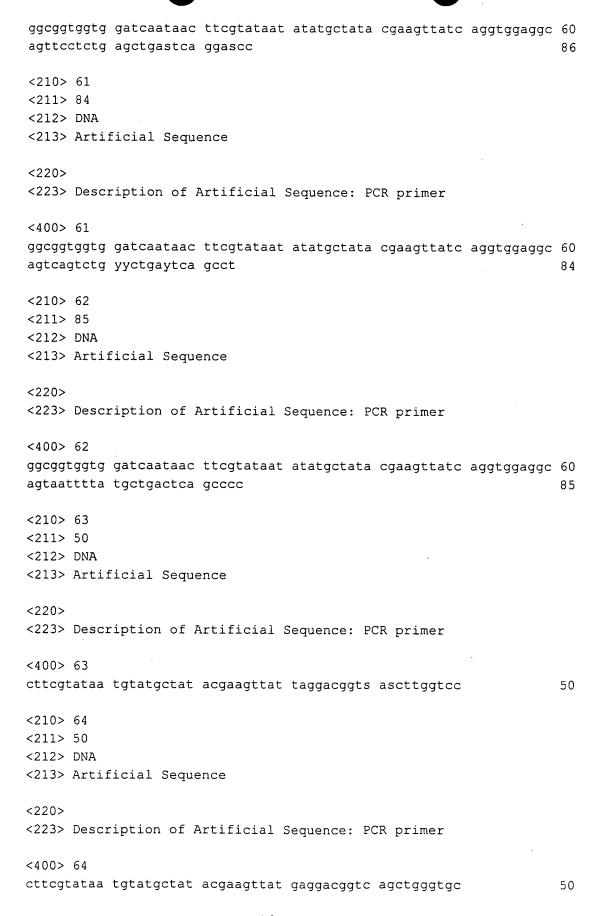
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| gaaacgacac tcacgcagte tc | 82 | |
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| agtcagtctg tsbtgacgca gccgcc | 86 |
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